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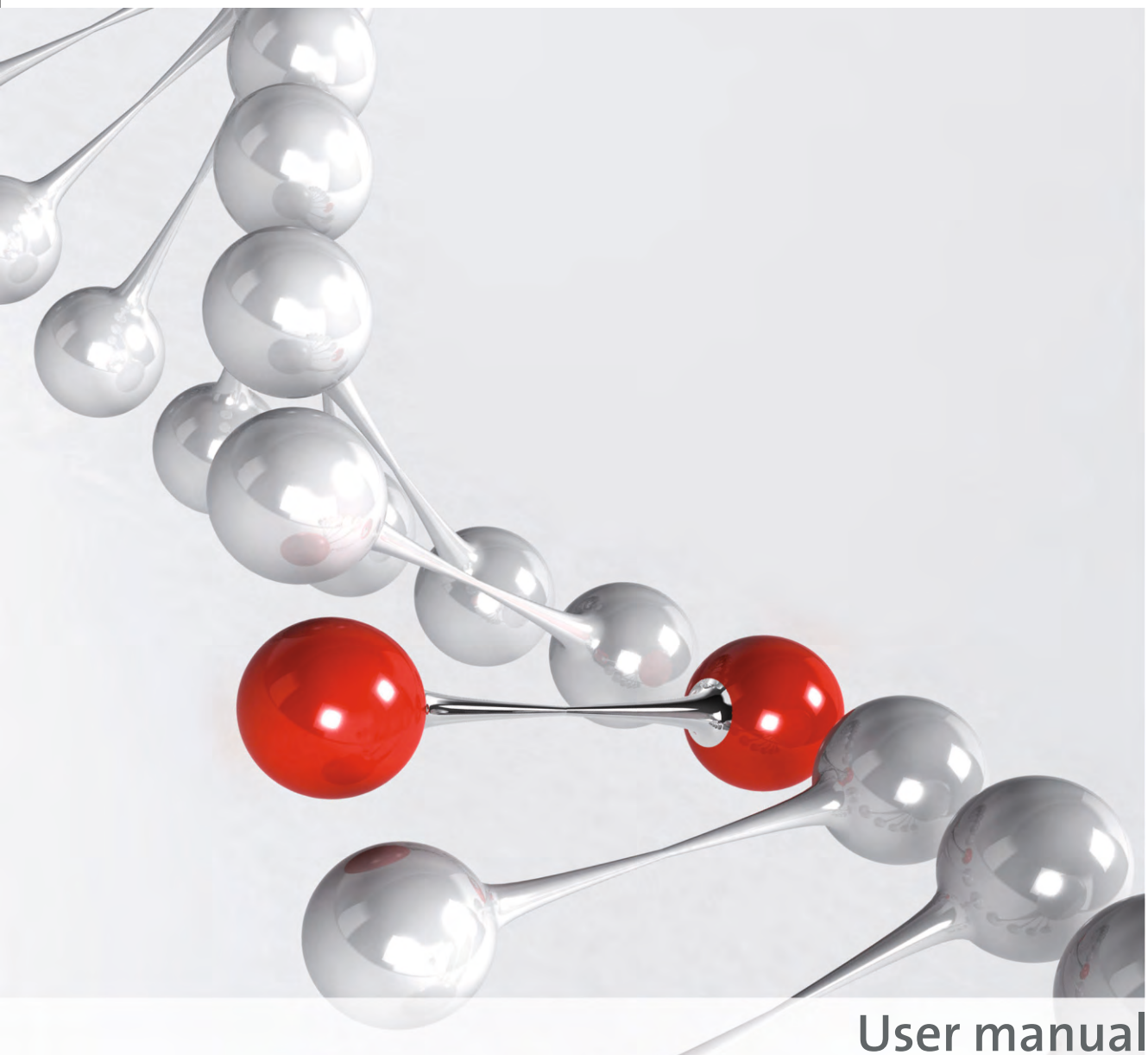

User manual
InviMag® SalivaGene DNA Kit/ KFmL
for use on KingFisher®mL, Thermo Fisher Scientific

for automated purification of total DNA from SalivaGene stabilized clinical swab (mouth brushes) & saliva sample with magnetic beads

REF 2435160X00 STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® SalivaGene DNA Kit/ KFmL

The **InviMag® SalivaGene DNA Kit/ KFmL** is used to stabilize and purify human and/or bacterial genomic DNA quantitatively by use of magnetic beads on the KingFisher® mL instrument.
The **InviMag® SalivaGene DNA Kit/ KFmL** is a modular system using a stabilization solution for collection, stabilization, storage and transportation of saliva material without degradation of DNA for at least 12 month with a very efficient isolation method for high quality DNA.

The kit is neither suitable for the isolation of DNA from stool samples, fungi nor for purification of total RNA.

Trademarks: InviMag®, SalivaGene®, Invisorb®, PSP®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag®, SalivaGene®, Invisorb® and PSP® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit content of the InviMag® SalivaGene DNA Kit/ KFmL

Store dissolved Proteinase K and Lysozyme at -20°C!
Store all other kit components at room temperature (RT)!

	15 extractions	75 extractions	300 extractions
Catalogue No.	2435160100	2435160200	2435160400
Lysozyme	for 500 µl working solution	for 2 x 1 ml working solution	for 7 ml working solution
Binding Buffer A	4 x 1 ml (ready to use)	9 ml (final volume 30 ml)	24 ml (final volume 80 ml)
MAP Solution B	0.5 ml	2 x 1 ml	7 ml
Proteinase K	for 500 µl working solution	for 2 ml working solution	for 7 ml working solution
Wash Buffer I	7,5 ml (final volume 15 ml)	30 ml (final volume 60 ml)	80 ml (final volume 160 ml)
Wash Buffer II	18 ml (final volume 60 ml)	2 x 18 ml (final volume 2 x 60 ml)	2 x 60 ml (final volume 2 x 200 ml)
Elution Buffer	2 ml	30 ml	80 ml
KingFisher mL Tip Combs	3	15	4 x 15
KingFisher mL Tube Strips	15	5 x 15	20 x 15
1.5 ml Receiver Tubes	15	5 x 15	20 x 15
Manual	1	1	1
Initial steps	Add 500 µl ddH_2O to the tube with **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C! Add 500 µl ddH_2O to the tube with **Lysozyme** mix thoroughly and store the tube at -20°C! Add 7.5 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap. Add 42 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap.	Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 2 ml ddH_2O to the tube with **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C! Add 1 ml ddH_2O to each tube with **Lysozyme**, mix thoroughly and store the tube at -20°C! Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap. Add 42 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap.	Add 56 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 7 ml ddH_2O to the tube with **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C! Add 7 ml ddH_2O to the tube with **Lysozyme**, mix thoroughly and store the tube at -20°C! Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap. Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap.

Kit content of the InviMag® SalivaGene DNA Kit/ KFmL / w/o plastic

Store dissolved Proteinase K and Lysozyme at -20°C!
Store all other kit components at room temperature (RT)!

	15 extractions	75 extractions	300 extractions
Catalogue No.	2435160150	2435160250	2435160450
Lysozyme	for 500 µl working solution	for 2 x 1 ml working solution	for 7 ml working solution
Binding Buffer A	4 x 1 ml (ready to use)	9 ml (final volume 30 ml)	24 ml (final volume 80 ml)
MAP Solution B	0.5 ml	2 x 1 ml	7 ml
Proteinase K	for 500 µl working solution	for 2 ml working solution	for 7 ml working solution
Wash Buffer I	7,5 ml (final volume 15 ml)	30 ml (final volume 60 ml)	80 ml (final volume 160 ml)
Wash Buffer II	18 ml (final volume 60 ml)	2 x 18 ml (final volume 2 x 60 ml)	2 x 60 ml (final volume 2 x 200 ml)
Elution Buffer	2 ml	30 ml	80 ml
Lysozyme	for 500 µl working solution	for 2 x 1 ml working solution	for 2 x 1 ml working solution
MAP B	500 µl	2 x 1 ml	2 x 1 ml
1.5 ml Receiver Tubes	15	5 x 15	20 x 15
Manual	1	1	1
Initial steps	Add 500 µl ddH_2O to the tube with **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C! Add 500 µl ddH_2O to the tube with **Lysozyme** mix thoroughly and store the tube at -20°C! Add 7.5 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap. Add 42 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap.	Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 2 ml ddH_2O to the tube with **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C! Add 1 ml ddH_2O to each tube with **Lysozyme**, mix thoroughly and store the tube at -20°C! Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap. Add 42 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap.	Add 56 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 56 ml 98 -100% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 7 ml ddH_2O to the tube with **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C! Add 7 ml ddH_2O to the tube with **Lysozyme**, mix thoroughly and store the tube at -20°C! Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap. Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap.
Plastic to be supplied by user (see order information)			
KingFisher mL Tip Combs	3	15	4 x 15
KingFisher mL Tube Strips	15	5 x 15	20 x 15

Content of SalivaGene® Stabilization Sets

Collection Sets	5 sets	50 sets
SalivaGene® Collection Module II	1035210600	1035210700
DNA Stabilizer (2 ml SalivaGene® DNA Stabilizer) prefilled in a Transport Tube	5 pieces	50 pieces
SalivaGene® Collection Tube, provided as Set	5 pieces	50 pieces
SalivaGene® Swab Collection Tube prefilled with DNA Stabilizer (500 µl SalivaGene DNA Stabilizer)	5 pieces	50 pieces
SalivaGene® Buccal Swab	1035230100	1035230200
Buccal Swabs	5 pieces	50 pieces
SalivaGene® Swab Collection Tube prefilled with DNA Stabilizer (500 µl SalivaGene DNA Stabilizer)	5 pieces	50 pieces

Symbols

Manufacturer

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit components, except the dissolved **Proteinase K** and the **Lysozyme** of the **InviMag® SalivaGene DNA Kit/ KFmL** should be stored sealed and dry at room temperature and are stable for at least 12 months under these conditions.

Dissolved **Proteinase K** and **Lysozyme** must be stored at -20°C. Repeated freezing and thawing cycles will reduce the activity of **Proteinase K** and **Lysozyme** dramatically. Dividing of dissolved **Proteinase K** and **Lysozyme** into aliquots and storage at -20°C is recommended.

Wash Buffer I and **II** charged with ethanol should be appropriate sealed and stored at room temperature.

The **MAP B** (magnetic beads) should be stored at 2-8°C.

Before use make sure that all components have room temperature. If any precipitates are visible within the provided solutions solve them by carefully warming up to 30°C.

Room temperature (RT) is defined as range from 15-30°C.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® SalivaGene DNA Kit/ KFmL** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® SalivaGene DNA Kit/ KFmL** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® SalivaGene DNA Kit/ KFmL** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany +49-(0)30-9489-2901/ 2910
from abroad +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **InviMag® SalivaGene DNA Kit/ KFmL** is suitable for reproducible purification of total DNA from up to 500 µl stabilization media of saliva samples in forensic, human-identity, genetic, biosecurity and pathogen analyses. The purification is very efficient and the isolated DNA performs well in downstream applications, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

The system consists of two modules, one collection set and one extraction module, useable independently or in combination.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from serum, plasma, fungi, parasites nor for the isolation and purification of total RNA.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® SalivaGene DNA Kit/ KFmL** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® SalivaGene DNA Kit/ KFmL** to which they apply, are listed below as follows:

Wash Buffer I

warning
H302-312-332-412 EU032 P273

Proteinase K:

danger
H315-319-334-335 P280-305-351-338-310-405

SalivaGene® DNA Stabilizer

danger
H319 P305-351-338

H319:	Causes serious eye irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H319:	Causes serious eye irritation
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P273:	Avoid release to the environment.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA:	**1 – 352 – 323 – 3500**
in USA :	**1 – 800 – 535 – 5053**

Product characteristic of the PSP® SalivaGene DNA Kit /KFmL

Starting material	Yield	Time	Ratio
500 µl of stabilization buffer (SalivaGene DNA Stabilizer) saliva mix 1:1	up to 10 µg (depends on kind of starting material)	23 min (without lysis)	A_{260} : A_{280} 1.7 – 2.0

The **InviMag® SalivaGene DNA Kit/ KFmL** uses the **PSP® Technology** (Preanalytical-Sample-Processing) a patented cost efficient technology and is a modular designed system for collection, stabilization, storage and transportation of saliva samples with DNA purification. The system combines the use of a prefilled **SalivaGene® Collection Tube** for saliva sample collection, the storage and stabilization of saliva specimen without any degradation of the DNA during transportation, including the prelysis of bacteria and a very efficient and fast isolation (about 23 min) of high quality total DNA from up to 500 µl mixture using the KFmL instrument. The kit is designed for isolation of DNA from host organism as well as for isolation of DNA from pathogen microorganisms.

After sample collection, the saliva sample is transferred in the **SalivaGene® Collection Tube** prefilled with **SalivaGene® DNA Stabilizer.** The raw material is lysed under these specific conditions. The **SalivaGene® DNA Stabilizer** causes an inactivation of DNases and thereby prevents the degradation of DNA, preserves the microorganism titer and prelyses bacteria. The transport of the stabilized DNA can be carried out in the **SalivaGene® DNA Stabilizer** containing tubes without cooling during 6 weeks at ambient temperature. The sample can also be stored in this buffer at -20°C for a long-term storage. For the DNA extraction process, an aliquot or the whole volume is used. The residual sample can be stored and used for further extractions.

A 500 µl aliquot of the stabilized sample is used in the **PSP® SalivaGene DNA** isolation procedure. At first, the proteins are degraded with Proteinase K, followed by a binding of the DNA to magnetic beads while contaminants, like potent inhibitors and proteins are rejected and washed-out, leaving the pure DNA to be eluted in either water or the buffer provided within the kit.

For reproducible and high yields appropriate sample storage is essential. Yields may be varying from sample to sample depending on factors such as the health of the donor, patient medication or sample storage conditions.

The procedure neither requires a phenol / chloroform extraction nor an alcohol precipitation. Furthermore, the kit requires only minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedure is designed to prevent sample-to-sample cross-contaminations.

Due to the high purity, the isolated genomic DNA is ready to use for a broad panel of downstream applications (see below) or can be stored at –20°C for subsequent use.

- PCR*, Real Time PCR Analysis*, RFLP / AFLP*- Analysis
- Restriction Enzyme Digestion
- HLA-Typing
- Pathogen Detection

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **InviMag® SalivaGene DNA Kit/ KFmL** cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Sampling and sample storage

Starting material

The amount of starting material used in **InviMag® SalivaGene DNA Kit/ KFmL** procedure can vary slightly, depending on the amount of DNA present in the sample. Specific guidance for starting amounts is given in the individual protocols.
The protocol works with aliquots of the transport media, in which fresh saliva samples are collected. The DNA in SalivaGene® stabilized samples is stable for 1 year at room temperature within the expiry date.The aliquots of the stabilization buffer may be processed on the same day as collection or stored for future processing. For long-term storage store the sample at -20°C.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Principle and procedure

The **InviMag® SalivaGene DNA Kit/ KFmL** procedure comprises the following steps:
- Collection of the material using a **SalivaGene® Collection Set**
- Lysis under non-chaotropic conditions at elevated temperature in Stabilization Buffer (**SalivaGene® DNA Stabilizer**)
- Protein digestion (**Proteinase K**)
- Binding of the genomic DNA to magnetic beads (**MAP B**)
- Washing of the beads and elimination of contaminants and ethanol
- Elution of pure genomic DNA

This manual contains 2 protocols, according to the different requirements of the starting materials used.

Lysis
Samples are lysed under non-chaotropic conditions at elevated temperature in the Stabilization Buffer (**SalivaGene® DNA Stabilizer**).

Protein digestion
Proteins will be digested in the lysed samples in the presence of **Proteinase K**.

Binding genomic DNA
By adding **Binding Buffer A** to the lysate, optimal binding conditions are adjusted.
Each lysate is then applied to magnetic beads and the genomic DNA is bound to the beads.

Removing residual contaminations
Contaminants are efficiently washed away using **Wash Buffer I and II**, while the genomic, mitochondrial and bacterial DNA remains bound to the magnetic beads.

Elution of pure genomic DNA
The isolated DNA is eluted from the magnetic beads using **Elution Buffer** and can be used in different downstream applications.

Yield and quality of DNA

The amount of purified DNA in the **InviMag® SalivaGene DNA Kit/ KFmL** procedure from different samples depends on the sample type, sample source, transport, storage and age. Yield and quality of isolated DNA are suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed accordingly to the manufacturer's specifications.

Important points before starting a protocol

General handling notes

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipette tips between liquid transfers. To avoid cross-contaminations, we recommend the use of aerosol-barrier pipette tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar airflow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

Before starting a run, equilibrate all reagents to room temperature. Where necessary, gently mix and redissolve any precipitates by warming up to 30°C. Swirl gently to avoid foaming.

1. Add the needed amount of ddH_2O to reaction tube with **Proteinase K** (see below). Vortex for 5 s. Repeat this step for the **Lysozyme** using **Lysozyme Buffer**.
2. Add the needed amount of ethanol to **Wash Buffer I** and **Wash Buffer II**

If a KFmL instrument is used for isolation the protein digestion has to be done on an additional themocycler: Adjust a thermomixer to 56°C (human sample). If bacterial DNA should be isolated a previous incubation step at 37°C is needed.

15 DNA extractions:
Add 500 µl ddH_2O to the tube with **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C! Add 500 µl ddH_2O to the tube with **Lysozyme** mix thoroughly and store the tube at -20°C! Add 7.5 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap. Add 42 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap
75 DNA extractions:
Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 2 ml ddH_2O to the tube with **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C! Add 1 ml ddH_2O to each tube with **Lysozyme**, mix thoroughly and store the tube at -20°C! Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap. Add 42 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap.
300 DNA extractions:
Add 56 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 7 ml ddH_2O to the tube with **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C! Add 7 ml ddH_2O to the tube with **Lysozyme**, mix thoroughly and store the tube at -20°C! Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap. Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap

Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.stratec.com under each STRATEC Molecular kit and kit component.

- Thermomixer (56°C (human, bacteria), 37°C (bacteria only))
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortexer
- ddH_2O
- 96-100% ethanol
- 1.5 ml reaction tube(s)
- Isopropanol

*The **InviMag® SalivaGene DNA Kit/ KFmL** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

*** Possible suppliers for Isopropanol:**

Fa. Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Fa. Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

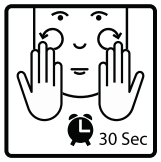
Fa. Sigma
2-Propanol
Order no. 59304-1L-F

PSP®-Treatment with SalivaGene® Collection Module II

Intended Use: Collection of human saliva samples for DNA stabilization and extraction (using the SalivaGene DNA Extraction Kits).
The DNA in SalivaGene stabilized samples is stable for 1 year at room temperature within the expiry date. The product is intended for use under supervision of professionals only, such as technicians, physicians and biologist trained in molecular biological techniques.

Contents: Collection tube, Stabilizer tube. The stabilizer tube contains approx. 2 ml of liquid Saliva DNA Stabilizer reagent.

Limitations of the procedure: Do not eat, drink, smoke or chew gum at least 30 min prior to saliva collection. Collecting 2 ml of saliva may require several minutes. Putting some grains of sugar on the tongue helps to produce saliva and does not interfere with the stabilization.

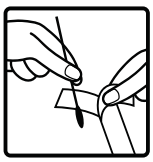
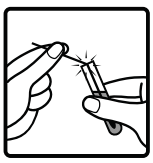
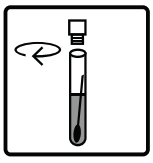
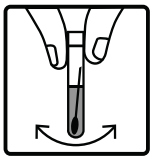
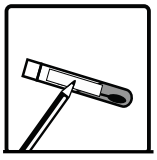
1.	Unscrew lid from Collection Tube and put it aside for further use.
2. 	Rub cheeks against teeth intensely for 30 sec.
3.	Collect saliva to indicated fill level, avoid making and measuring air bubbles.
4.	Unscrew and discard lid from Stabilizer Tube. Pour Saliva DNA Stabilizer reagent into Collection Tube.
5.	Screw lid tightly onto the Collection Tube again.
6.	Shake tube for 15 sec to mix saliva and Saliva DNA Stabilizer reagent.

PSP-Treatment of SalivaGene® Buccal Swab

Intended Use: Collection of human oral samples for DNA stabilization and extraction (using the SalivaGene DNA Extraction Kits).
The DNA in SalivaGene stabilized samples is stable for 1 year at room temperature within the expiry date. The product is intended for use under supervision of professionals only, such as technicians, physicians and biologist trained in molecular biological techniques.

Contents: Swab, Stabilizer tube, label for donor description. The Stabilizer tube contains approx. 2 ml of liquid DNA Stabilizer reagent.

Limitations of the procedure: Do not eat, drink, smoke or chew gum at least 30 min prior to swab collection. Ensure the swab tip does not come into contact with any surface prior to collection. Put the Stabilizer tube upright to prevent the liquid inside the tube from spilling. Be sure to move the swab over the entire cheek and to moisten it with saliva.

1. 	Open swab package and remove swab without touching the tip.
2.	Insert swab into the mouth and rub swab tip firmly against the inner cheeks for about 30 seconds on each side.
3.	Unscrew lid from Stabilizer tube and put it aside for further use. Insert swab into the Stabilizer tube.
4. 	Break swab tip at breaking point. Discard the broken handle part.
5. 	Screw lid tightly onto the Stabilizer tube again.
6. 	Gently shake tube for 15 sec to mix buccal cells and DNA Stabilizer reagent.
7. 	Label Stabilizer tube with donor name and collection date using provided label.

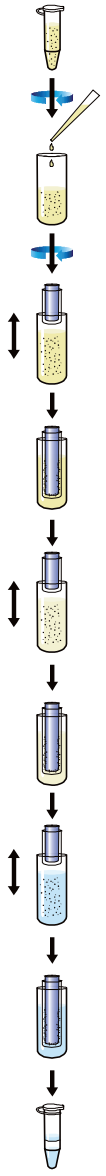
Scheme of the InviMag® SalivaGene DNA Kit/ KFmL

Please carefully read protocols prior starting with the preparation

Transfer 500 µl stabilized sample into a 1.5 ml reaction tube and add the appropriate lysis reagent (PK and/or Lysozyme, see **(1a)** or **(1b)**

During lysis, prefill the Tube Stripes with the needed buffers as described below.

Binding Tube A:	Add **200 µl Binding Buffer A** *(follow preparing instructions)* and **20 µl MAP B**
Washing Tube B:	Add **500 µl Wash Buffer I**
Washing Tube C:	Add **600 µl Wash Buffer II**
Washing Tube D:	Add **600 µl Wash Buffer II**
Elution Tube E:	Add **200 µl Elution Buffer**



(1a) Bacterial DNA: Transfer 500 µl stabilized sample into a 1.5 ml reaction tube and add 20 µl Lysozyme. Incubate for 10 min at 37°C while continuously shaking. Afterwards add 20 µl Proteinase K and incubate for 20 min at 56°C while continuously shaking. Continue with step **(2)** of the manual.
During lysis, prefill all Tube Stripes with required buffers and appropriate volumes.

(1b) Human DNA:
Transfer 500 µl stabilized sample into a 1.5 ml reaction tube, add 20 µl Proteinase K and incubate for 20 min at 56°C while continuously shaking. Continue with step **(2)**. During lysis, prefill all Tube Stripes with required buffers and appropriate volumes.

(2) After the lysis step, transfer the whole sample from step **(1a)** or **(1b)** into the Tube Stripe A (containing 200 µl **Binding Buffer A** and 20 µl **MAP B**) and select the program "**InviMag Salivagene DNA KFmL**"

DNA binds to magnetic particles

Magnetic separation

Washing of the particle fixed genomic DNA

Magnetic separation

Elution of genomic DNA

Magnetic Separation

Pure DNA

Protocol I: Human DNA extraction from stabilized material

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction page: 10*

Lysis (external)

Transfer 500 µl of **Saliva / DNA Stabilizing Mixture** from the **SalivaGene® Collection Tube** into a 1.5 ml reaction tube (not provided).

Add 20 µl of **Proteinase K** and incubate for 20 min at 56°C while continuously shaking or alternatively flick the tube every 5 min.

Note: *If you have to archive a SalivaGene sample, e.g. for forensic purposes, store the residual sample not needed for preparation at -20°C. The sample is stable for several years under these conditions. Prepare the Tube Stripes with the appropriate buffers and volumes needed during the lysis process (see below for detailed information).*

During lysis, prepare the Tube Stripes with the provided buffers and appropriate volumes. See below for detailed information.

Important: *Mix the bottle with the* ***MAP B*** *by vigorously vortexing!*

Tube Stripe A:	Transfer 200 µl **Binding Buffer A** and 20 µl **MAP B** into Tube Stripe A
Tube Stripe B:	Transfer 500 µl **Wash Buffer I**
Tube Stripe C:	Transfer 600 µl **Wash Buffer II**
Tube Stripe D:	Transfer 600 µl **Wash Buffer II**
Tube Stripe E:	Transfer 200 µl **Elution Buffer**

Starting a run

After lysis, transfer the lysed sample into the Tube Stripe A containing 200 µl **Binding Buffer A** and 20 µl **MAP B**. Start the purification by selecting the assay file **"InviMag Salivagene DNA KFmL"** and press the "START" button.

The DNA will bind to the magnetic beads and will then be transported through the different washing solutions before the elution will take place in Tube Stripe E. The eluate can finally be transferred into the provided Receiver Tubes.

Protocol II: Bacterial DNA extraction from stabilized material

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction page: 10*

Lysis (external)

Transfer 500 µl **Saliva / DNA Stabilizing Mixture** from the **SalivaGene® Collection Tube** into a 1.5 ml reaction tube (not provided).

Add 20 µl of Lysozyme, vortex for 5 s and incubate the mixture for 10 min at 37°C while continuously shaking or flicking the tube from time to time. Afterwards add 20 µl Proteinase K and incubate for an additional 20 min at 56°C while continuously shaking or every 5 minutes flicking the tube.

Note: *If you have to archive a SalivaGene sample, e.g. for forensic purposes, just freeze the rest you don't use for preparation. The rest of the stabilized sample can be stored at –20°C for years.*

During lysis, prepare the Tube Stripes with the provided buffers and appropriate volumes. See below for detailed information.

Important: *Mix the bottle with the* ***MAP B*** *by vigorously vortexing!*

Tube Stripe A:	Transfer 200 µl **Binding Buffer A** and 20 µl **MAP B** into Tube Stripe A
Tube Stripe B:	Transfer 500 µl **Wash Buffer I**
Tube Stripe C:	Transfer 600 µl **Wash Buffer II**
Tube Stripe D:	Transfer 600 µl **Wash Buffer II**
Tube Stripe E:	Transfer 200 µl **Elution Buffer**

Starting a run

After lysis, transfer the lysed sample into the Tube Stripe A containing 200 µl **Binding Buffer A** and 20 µl **MAP B**. Start the protocol by selecting the assay file **"InviMag Salivagene DNA KFmL"** and press the "START" button.

The DNA will bind to the magnetic beads and will be transported through the different washing buffers before the elution is performed in Tube Stripe E.

The eluate can finally be transferred into the provided Receiver Tubes after the assay has been finished.

For self programming of the KFmL instrument

[Protocol Properties]

Software Version: BindIt 3.1
Name = InviMag Salivagene DNA KFmL
Protocol template version = 3.1
Instrument type = KingFisher mL
Creator = STRATEC Molecular GmbH
Description = KFmL protocol (Thermo Electron) for isolation of genomic, mitochondrial and/or bacterial DNA from saliva samples (human) stored in Saligene® Stabilization Tubes with InviMag® SalivaGene DNA Kit/ KFmL.
Kit = InviMag® SalivaGene DNA Kit/ KFmL

[Layout Data]

Tray/plate type: KingFisher tubestrip 1000 µl

Tube A (Binding)
Reagents:
Name: Lysed Sample
Volume [µl]: 520
Type: Sample

Name: MAP B
Volume [µl]: 20
Type: Reagent

Name: Binding Buffer A
Volume [µl]: 200
Type: Reagent

Tube B (Wash 1)
Reagents:
Name: Wash Buffer I
Volume [µl]: 500
Type: Reagent

Tube C (Wash 2)
Reagents:
Name: Wash Buffer II
Volume [µl]: 600
Type: Reagent

Tube D (Wash 3)
Reagents:
Name: Wash Buffer II
Volume [µl]: 600
Type: Reagent

Tube E (Elution)
Reagents:
Name: Elution Buffer
Volume [µl]: 200
Type: Reagent

[Steps]

Tip1: KingFisher ml tip comb

Binding (Plate: Salivagene (A) – Binding)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:05:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 4
Collect time [s]: 3

Wash 1 (Plate: Salivagene (B) - Wash 1)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:30
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Wash 2 (Plate: Salivagene (C) - Wash 2)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Wash 3 (Plate: Salivagene (D) - Wash 3)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Medium

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

End of step:
Postmix: No

Collect count: 3
Collect time [s]: 2

Drying (Plate: Salivagene (D) - Wash 3)
Dry time [hh:mm:ss]: 00:03:30
Tip position: Outside well / tube

Elution Plate (Salivagene (E) – Elution)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Medium

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:10:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 4
Collect time [s]: 3

Bead Removal (Plate: Salivagene (D)-Wash3)
Release time [hh:mm:ss]: 00:00:30
Release speed: Fast

Troubleshooting

Problem	Cause	Comments and suggestions
amount of DNA	insufficient cell lysis due to decreased **Proteinase K / Lysozyme** activity	repeat the DNA purification procedure with a new sample and freshly prepared **Proteinase K / Lysozyme** stock solution. be sure to make aliquots and store them at -20°C
	insufficient lysis due to insufficient mixing with lysis buffer **- SalivaGene® DNA Stabilizer**	repeat the DNA purification procedure with a new sample. Be sure to mix the sample and **SalivaGene® DNA Stabilizer** immediately
	low percentage alcohol used	repeat purification procedure with a new sample
	incomplete elution	increase incubation time with **Elution Buffer** to 5-10 min or increase the elution volume to 200 µl. This will however reduce the final DNA concentration. Make sure to adjust modification in the KingFisher software
	pH of water incorrect (acidic)	low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use **Elution Buffer** (contains 10 mM Tris-HCl but no EDTA)
degraded or sheared DNA	incorrect storage of starting material	ensure the sample is harvested and stored correctly
	old material	avoid repeated thawing and freezing cycles of the material. Old samples often contain degraded DNA
problems with subsequent applications (e.g. in PCR)	ethanol in the eluted DNA	verify if the recommended dry time was reached increase drying time for the elimination of ethanol in the run file
	salt in the eluate	**Wash Buffer** should be stored and used at RT make sure that no precipitates are visible within the Washing Buffers. If any precipitations are visible, dissolve them by carefully warming up to 30°C
	reduced sensitivity of amplification reaction	adjust the volume of eluate added as template in the amplification reaction
A_{260}/A_{280} ratio for purified DNA is low	insufficient cell lysis and protein degradation due to decreased **Proteinase K / Lysozyme** activity	see above under “low amount of DNA”
	insufficient lysis due to incomplete mixing with lysis buffer	see above under “ low amount of DNA”

Appendix

KingFisher Software 3.1

The KingFisher Software 3.1 was used to create assay files for the KFmL, KF96 and KFflex96 instruments. The respective assay file can either be transferred onto the KingFisher workstation or be started directly from within the BindIt software. Keep in mind that directly run assay files are not stored in the workstation memory!

Note: *When creating assay files for usage with KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.1 for assay development as this software version includes the correct adjustments for this plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 workstations to ensure the best purification result.*

PC requirements for KingFisher Software 3.1

PC requirements	
Interface	Serial communication port via a RS-232 full duplex interface
Supported operating systems	Microsoft Windows 2000 Microsoft Windows XP Professional
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 700 MHz recommended
Memory	220 MB RAM recommended
Serial ports available	1
Pointing device	Mouse or equivalent is necessary
CD-ROM drive	1
Monitor / color settings	SVGA monitor with at least 1024 x 768 resolution and at least a 16-bit color environment
Service packs installed	Microsoft Windows 2000: Service Pack 4 (or greater) Microsoft Windows XP Professional: Service Pack 2 (or greater)
Browser	Microsoft Internet Explorer 6.0 (or greater) installed

If you do not have the correct Service Packs installed, you can download them from the Microsoft web pages: http://www.microsoft.com/

General notes on handling DNA

Starting material
This kit is designed for extraction of DNA from saliva and swabs. These materials show big variation in DNA contents. The purification of some apoptotic DNA is normal.

Nature of DNA
The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, and long-template PCR.

Storage of DNA
A working stock of DNA can be stored at 2 – 4˚C for several weeks. For long term storage DNA should be stored at - 20˚C, but storing at – 20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect its stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA
Avoid over drying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

Ordering information

Product	Package size	Catalogue No.
InviMag® SalivaGene DNA Kit /KFmL	15 preps	2435160100
InviMag® SalivaGene DNA Kit /KFmL	75 preps	2435160200
InviMag® SalivaGene DNA Kit /KFmL	300 preps	2435160400
InviMag® SalivaGene DNA Kit /KF96 /KFflex 96	1x 96 preps	7435300100
InviMag® SalivaGene DNA Kit /KF96 /KFflex 96	5x 96 preps	7435300200
PSP® SalivaGene DNA Kit	5 preparations	1035200100
PSP® SalivaGene DNA Kit	50 preparations	1035200200
PSP® SalivaGene DNA Kit	250 preparations	1035200300
SalivaGene® Collection Module II	5 container	1035210600
SalivaGene® Collection Module II	50 container	1035210700
SalivaGene® Buccal Swab	5 pieces	1035230100
SalivaGene® Buccal Swab	50 pieces	1035230200

Ordering information (KingFisher mL and consumables from Thermo Scientific)

Cat.no	Description
5400050	KingFisher mL, Magnetic Particle Processor, 100-240 V, 50/60 Hz
97002111	KingFisher mL tip comb, 800 pcs
97002121	KingFisher mL tube, 900 pcs (20x45 pcs)
97002131	KingFisher mL Combi 60 (tubes and tip combs for 60 samples)
97002141	KingFisher mL Combi 240 (tubes and tip combs for 240 samples)

Possible suppliers for Isopropanol:

Fa. Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Fa. Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Fa. Sigma
2-Propanol
Order no. 59304-1L-F

stratec molecular

STRATEC Molecular GmbH
Robert-Rössle-Str. 10
13125 Berlin, Germany

Phone: +49 30 94 89 29 01
Fax: +49 30 94 89 29 09
E-mail: info.berlin@stratec.com

www.stratec.com

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